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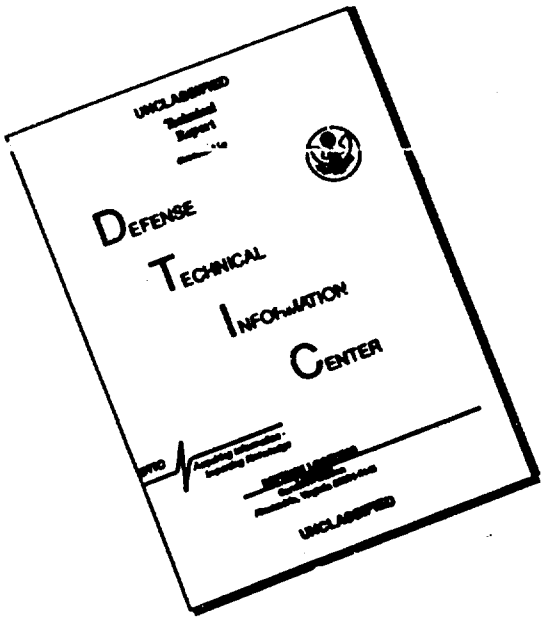

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NEUTRALIZATION BY VARIOUS METABOLITES OF THE INHIBITORY EFFECT OF GUANIDINE ON THE DEVELOPMENT OF POLIOVIRUS

[Following is a translation of a report by A. Lwoff and M. Lwoff of the Microbial Physiology department of the Pasteur Institute, (presented by J. Trefouel) in the French-language journal Comptes Rendus de l'Academie des Sciences, Paris (Reports of the Academy of Sciences, Paris), Vol 259, Paris, 27 July 1964, pages 948-952.]

The development of type I poliovirus in Earle's medium is totally inhibited by ~~2×10^{-4}M~~ .002M guanidine. Choline, methionine and valine neutralize this effect the mechanism of the action of these antiguanidines is discussed ~~below~~.

Guanidine-requiring strains of poliovirus do not develop in the absence of guanidine which can be replaced by substance possessing this radical[1]. In cells infected with guanidine[2]requiring poliovirus, the viral RNA-replicase does not appear in the absence of guanidine, which is thought to contribute to the achievement of a tertiary or quarternary structure of the enzyme[3].

The original strains of poliovirus are inhibited by guanidine, which prevents the formation of the viral RNA-replicase[4]. If cellular metabolites intervene in this information, as they do in the structure of phosphorylase or in glutamic acid dehydrogenase, the blockage of viral development by guanidine is perhaps due to an inhibitor-effecter competition. We have tried to find evidence for substances capable of diminishing the inhibitory effect of guanidine.

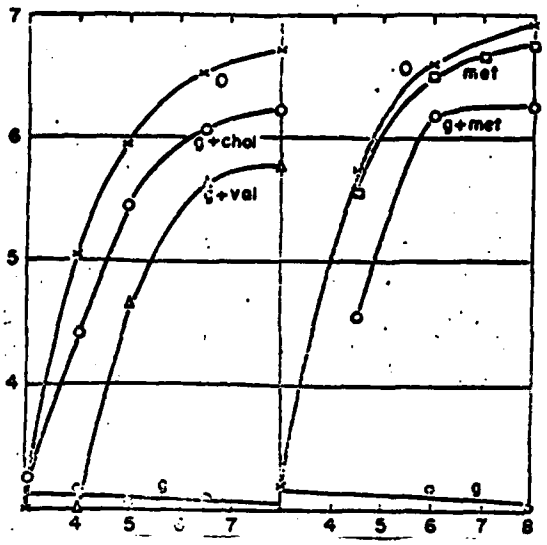
KB cells cultivated in lactalbumin medium enriched with autolysate of yeast and serum were washed, infected with poliovirus of type I (original strain LSc 2 ab of Sabin), and after elimination of non-adsorbed virus, were put in suspension in nutritive media containing extract of bovine embryo at a final concentration of 2%. The kinetics of viral development during the course of one full cycle was established by counting the cell units forming plaques.

The inhibitory action of guanidine varies according to the type of medium used. A concentration of 3×10^{-4}M guanidine blocks development in Earle's medium (yield 0.1 to 0.01% of the control). In medium consisting of lactalbumin + hydrolysate of yeast, the inhibition is much less marked and above all more irregular. The addition to Earle's medium of yeast autolysate at a concentration of 0.3 to 0.6% permits a respective yield of [illegible]. Finally, in modified Eagle's medium, called "4EM" (concentration of vitamins and amino acids four times that of the original mixture), the yield is 100%: there is no inhibition in this case.

We then studied the effects of the constitutents of Eagle's medium on the development of poliovirus in Earle's medium with added guanidine, the concentration of the various substances being the same as in the "4EM" medium. The results are shown in the figure below and the table gives the percentage yield as compared to the controls.

Guanidine 3×10^{-4}M	0.1 - 0.01
" + L(+)-valine 1.5×10^{-3}M	11
" + L(-)- methionine 4×10^{-4}M	21
" + choline 3×10^{-5}M	30

The L(-)-leucine at 1.5×10^{-3} showed a feeble activity (Rdt 1%). Similarly, L(+)-alanine at 4×10^{-3} gives feeble activity (Rdt 4%), but only in the presence of the vitamins in Eagle's medium. The other constituents of the medium (group B vitamins, hypoxanthine, amino acids other than those already mentioned) are devoid of antiguanidine activity under the conditions of these experiments. However, "4EM" medium, while antiguanidine in its full complement, is a mixture of inhibitory and anti-inhibitory substances. It appeared necessary thus to examine the effects of the constituents at varying concentrations to exclude the possibility of inhibitory action caused only by the isolated



Kinetics of the poliovirus development at 36° in Earle's medium + embrgonic extract.

Abscissas: Hours after infection; ordinates: units forming plaques per an illiliter, in logarithmic coordinates. O = control; g = guanidine, $3x10^{-4}M$; chol-cholmic $3.3x10^{-5}M$; meth= L(-) methionine $4x10^{-4}M$; val = L(t)- valine $1.5x10^{-3}M$.

components. Note that methionine, which is antiguanidine in Earle's medium at a concentration of $4 \times 10^{-4}M$, completely inhibits poliovirus development at a concentration of $2 \times 10^{-3}M$.

Thus certain constituents of cell culture media diminish the inhibitory action of guanidine, and the problem of determining the mechanism of this effect is one which quite naturally comes to mind. Two hypotheses among others can be envisaged. The first is that antiguanidine substances combine with the guanidine and form a less active complex. KB cells can, for example, methylate the guanidine in the presence of an excess of choline or methionine. This supposition is not in contradiction with the fact that guanidine is not metabolished by the animal organism under normal conditions. The second hypothesis is that antiguanidines represent either effectors or substances intervening in the synthesis of effectors of viral development. These effectors

play the same role for the susceptible strain as does guanidine for the guanidine-requiring strain. Current experiments should permit us to choose between the two hypotheses. It was known that one substance favored the development of poliovirus: semi-carbazide[6]. This compound augments the rate of development in Earle's medium, doubling time 9 min. instead of 14, and also the yield of units forming plaques. It does not have, as shown in the table below, an abilityto neutralize the inhibitory effect of guanidine, nor does it affect the antiguanidine action of choline. The yields are shown below (the numbers of represent the percentage yield as compared to the control):

Semi-carbazide 2.10^{-2}M	333
Guanidine 3.10^{-4}M	0.03
Semi-carbazide + guanidine	0.07
Choline $3.3,10^{-3}$M	72
Choline + guanidine	8
Semi-carbazide + choline	445
Semi-carbazide + choline + guanidine	110

Whatever the mechanism may be of the action of choline, methionine and valine, it does not remain unless normal cellular metabolites neutralize the inhibitory effect of guanidine on the sensitive strain's development. It was previously known that normal cellular metabolites, such as arginine, glycine, creatine and glutamine assure the development of the guanidine-requiring strain. It was also known that normal cellular metabolites, such as arginine and glycine, inhibit the development of the guanidine-sensitive strain of poliovirus, while they are without effect on the guanidine-resistant strain[6]. Thus a number of essential metabolites influence the development of poliovirus in one way or another. Their action depends, of course, on the genetic composition of the virus [illegible words] of given genetic composition, on the intracellular concentration of the metabolites concerned. This is probably a function of the nature and physiologic state of the cells, of the constituents of the medium, the modifications of the medium provoked by cellular metabolism, that is to say, particular parts of the metabolism, of the cell concentration, the duration of the experiment and finally the perturbations of cellular metabolism caused by the viral infection itself. Any analysis of the results must take into consideration the multiplicity of factors and their possible interaction.

Finally, it is necessary not to forget, during the course of chemo-therapy assays <u>in vitro</u> and <u>in vivo</u>, that

essential metabolites can diminish or accentuate the effect of certain antiviral agents.

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Notes

1. A. Lwoff, M. Lwoff and A. Koch, Comptes rendus (Reports), Vol 257, 1963, page 4068.

2. H. J. Eggers, E. Reich and I. Tamm, Proc. Nat. Acad. sci. Vol 50, 1963, page 183-193.

3. A. Lwoff and M. Lwoff, Comptes rendus, Vol 256, 1963, page 5001.

4. D. Baltimore, H. J. Eggers, R. M. Franklin and I. Tamm Proc. Nat. Acad. Sci. Vol 49, 1963, page 843-849.

5. A. Lwoff and M. Lwoff, Comptes rendus, Vol 258, 1964, page 1924.

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